REMARKS

Claims 15-18 were added to more clearly define that which Applicants regard as the invention. Support for Claims 15-18 can be found in the specification. For example, page 3, lines 27-28 describe an embodiment in which the platform is a moveable platform. Entry of Claims 15-18 is respectfully requested.

In the Office Action dated November 2, 2006, the Examiner rejected claims 1, 3, 5-8, 10 and 12-14 under 35 U.S.C. § 103(a) as being unpatentable over Heidt (U.S. 5,089,229), Copeland (U.S. 5,654,200), Kerr (U.S. 5,075,079) or Rogers (U.S. 4,043,292) in view of Potter (U.S. 5,819,842). The Examiner stated that the primary references (Heidt, Copeland, Kerr and Rogers) "are directed to the automated processing of slides encompassing all of the claimed elements except for an individual sensor beneath each slide to control each slides [sic] temperature independently." (November 2, 2006 Office Action at 3.) As discussed more fully below, none of the primary references cited by the Examiner disclose plural heated surface areas, each heated by an electric heater thereunder, wherein each heated surface area is adapted to be in contact with and underlie a microscope slide bearing a biological sample.

Potter is said to teach "an apparatus for manipulation of biological samples on slides" and "an individual sensor below each slide that regulates the temperature of each individual slide." (Id.) In fact, Potter does not disclose such an apparatus because Potter is not directed to the analysis of biological samples on slides. Instead, as discussed below, Potter is directed to liquid samples contained in a well.

The Examiner has rejected Applicants' argument that there was no motivation for one of skill in the art of slide staining, at the time of the invention, to combine the heating system of Potter with the apparatus disclosed in Heidt, Copeland, Kerr or Rogers. In the most recent Office Action, the Examiner states that "Applicants' also argue the rejection of record is directed to a different method of intended use" and that "[t]hese remarks are not convincing because the method of intended use of an apparatus is of no patentable moment." A more accurate description of Applicants' position is that each reference cited by the Examiner must be considered in the context of the state of the art at the time of the invention. Applicants have presented evidence that the heating system of Potter was advantageous for the uses contemplated

by Potter (i.e., DNA or RNA amplification, enzyme reactions, and investigating rates of hybridization and melting of nucleic acids), but that there was no teaching, suggestion, or motivation for one of skill in the art to apply the heating system as described in Potter to the apparatuses disclosed in the primary references.

In view of his understanding of the teachings of the prior art, the Examiner concludes that the claimed invention is obvious.

1. Standard for Determining Obviousness

In the recent case *KSR Int'l Co. v. Teleflex, Inc.*, 127 S.Ct.1727 (2007), the Supreme Court articulated a flexible test for determining whether an invention was obvious over the prior art. The Examination Guidelines for Determining Obviousness Under 35 U.S.C. 103 in View of the Supreme Court Decision in *KSR International Co. v. Teleflex, Inc.*, (Federal Register Volume 72, No. 195, pp. 57526-57535, October 10, 2007) ("Guidelines for Determining Obviousness) states that to support an obviousness rejection based on the rationale that there is some teaching, suggestion, or motivation in the prior art that would have led one of ordinary skill to modify the prior art reference or to combine prior art reference teachings to arrive at the claimed invention, the Examiner must articulate the following:

> (1) a finding that there was some teaching, suggestion, or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings;
>
> (2) a finding that there was reasonable expectation of success; and
>
> (3) whatever additional findings based on the *Graham* factual inquiries may be necessary, in view of the facts of the case under consideration, to explain a conclusion of obviousness.

Guidelines for Determining Obviousness at page 57534.

The Guidelines for Determining Obviousness further highlights the Supreme Court's reaffirmation of *Graham v. John Deere*, 383 U.S. 1 (1966). In *Graham* the Supreme Court articulated four factual inquiries for determining obviousness.

> Under § 103, the scope and content of the prior art are to be determined; differences between the prior art and the claims at issue are to be ascertained; and the level of ordinary skill in the

> pertinent art resolved. Against this background, the obviousness or nonobviousness of the subject matter is determined. Such secondary considerations as commercial success, long felt but unsolved needs, failure of others, etc., might be utilized to give light to the circumstances surrounding the origin of the subject matter sought to be patented. As indicia of obviousness or nonobviousness, these inquiries may have relevancy.

Graham, 383 U.S. at 17-18.

Additionally, in *KSR*, the Supreme Court, citing *Graham*, warned against the use of hindsight bias in determining obviousness, stating that "[a] factfinder should be aware, of course, of the distortion caused by hindsight bias and must be cautious of arguments reliant upon *ex post* reasoning." *Id.* at 1742.

2. The Scope and Contents of the Prior Art and the Level of Ordinary Skill in the Art

As stated in the attached Declaration of Ron Zeheb, Ph.D., Under 37 C.F.R. § 1.132, at the time of the invention, all slide staining processes could be categorized as follows:

A. Routine Staining

Routine staining is performed as a batch process where all slides are treated the same. The slides are typically mounted in baskets that are dipped into buckets of solution. As such, they do not require random access dispensing systems as claimed. Further, they generally do not require heating.

B. Advanced Staining

There are three general categories of advanced staining, commonly known as special stains, immunohistochemistry, and *in situ* hybridization. At the time of the invention, one of ordinary skill would not have predicted the utility of plural heated surface areas, each heated by an electric heater thereunder and having a respective temperature sensor, in a random access dispensing assembly or in a method for processing biological samples mounted on microscope slides.

i. Special Stains

At the time of the invention, special stain techniques often required judgments on the part of the technician, such as color analysis. Namely, the technician dipped the slide in a chemical

or dye until the tissue elements acquired a certain specified color, as determined visually. Examples of special stain processes are presented in provided references Luna (1968) (Exhibit A), Prophet et al. (1992) (Exhibit B) and Bancroft and Stevens (1996) (Exhibit C). In these references, arrows with asterisks indicate steps in the procedures which must be performed visually and thus require user input. Because such techniques rely highly on the skills of the technician, and are considered an art, they had not been considered appropriate for automatic processing.

ii. Immunohistochemistry

The second type of advanced stain is immunohistochemistry. Apparatuses such as those disclosed in cited references Copeland and Rogers would have been seen by one of skill in the art as being particularly suited to immunohistochemical staining (also called immunostaining). (See e.g., Copeland, column 2, lines 30-39.) As practiced in 1994, immunohistochemical slides were either processed at room temperature (without the application of heat) or were heated to approximately body temperature. In either situation, all of the slides were processed at the same temperature, regardless of the particular histochemical stain. The automated slide stainers on the market by 1994 did one or the other. Examples of automated slide stainers without any heating capability were Fisher's Code-on and Shandon's Cadenza; whereas, Ventana's 320/ES immunohistochemical slide stainer (similar to the Copeland primary reference) heated all of the slides to approximately body temperature.

iii. *In situ* Hybridization

The third category of advanced staining is *in situ* hybridization (ISH). This type of stain requires temperatures that are much higher than body temperature, often in the 70-95°C range. However, the small volume of reagent probe typically used (approximately ten microliters) can rapidly evaporate at such temperatures. To prevent evaporation during ISH processing, the tissue section and the small amount of reagent/probe are sealed on the slide using a coverslip. The edges of the coverslip are sealed with, for example, rubber cement or nail polish. A system in which the sample must be sealed off from the outside environment is mechanically incompatible with a device that controls relative movement between a liquid dispenser and a platform, and that dispenses liquid reagents onto a microscope slide bearing a biological sample, such as in the claimed dispensing assembly. One of ordinary skill at the time of the invention

(1994) would not have considered a system with high temperature heating and a stringent requirement for preventing evaporation of an extremely low volume of reagent as compatible with an open dispensing system.

3. Teachings of the Cited References

A. Copeland

Copeland teaches an automated immunostaining apparatus having a reagent application zone and a reagent supply zone. The apparatus has a carousel slide support supporting a plurality of slide supports and a drive device that allows for consecutively positioning each of a plurality of slide supports in the reagent application zone (see Abstract). Copeland further describes an embodiment in which the apparatus has a "heating chamber means surrounding the slide support carousel for heating slides supported thereon to a predetermined temperature." (See column 3, lines 8-10.) Thus, Copeland teaches convective heating rather than contact (conductive) heating to heat the slides (column 3, lines 8-22). This means that the slides of Copeland are simply warmed to a common temperature by air that has been heated.

B. Rogers

Rogers teaches an apparatus in which slides are supported on a rotary carousel. Heat is provided using the typical approach of moving the slides into a heated chamber. Specifically, in Figure 4 of Rogers, the heating element 80 provides heat to air blown into the interior of the unit to heat the slides. Figure 6 discloses a radiant heater 90. The support elements 26 on the carousel (Figures 2 and 3 of Rogers) cannot be considered plural heated surface areas, each heated by an electric heater thereunder, because they do not provide heat to the slides. Rather, they simply support the slides within the heated environment.

C. Heidt and Kerr

In both Heidt and Kerr, instead of a reagent dropping onto a microscope slide bearing a biological sample, a drop of serum is dispensed onto various chemical analyte "slides," each of which is impregnated with a reagent that causes a color to develop upon reacting with substances in the serum. The analyte slides are heated to a common temperature by convective heating and are optically analyzed within the system.

The analyte slides in Heidt are described as follows

> When the 10 microliters [of serum] are forced out of the pipette tip, a drop will form and be suspended below the open end 310 of the tip. The pipette lifter assembly is then activated, which will cause the pipette tip 176 to be lowered until the drop touches the film portion 124 of the test slide, where upon, by capillary action, the sample serum will flow onto the analyte film portion of the test slide

(Column 23, lines 57-64).

The analyte slides in Kerr are described as follows:

> Each slide 28 includes a circular analysis or examination area 29 (FIGS. 7, 9 and 10) with an absorbent receiving surface 31 at a top portion 33 of the slide 28 for spotting with a fluid or serum sample. A translucent barrier strip 35 for preventing evaporation and blocking fluid drainage is provided across the analysis area 29 at a bottom surface 37 of the slide 28. Both the receiving surface 31 and the barrier strip 35 are respectively recessed from the top and bottom surfaces 33 and 37 of the slide 28.

(Column 5, line 67 through Column 6, line 5).

Based on these teachings, one of skill in the art would not have seen the apparatuses disclosed in Heidt and Kerr as pertaining to the field of microscope slide staining. Furthermore, even if one of skill in the art of slide staining would have considered either Heidt or Kerr, neither reference is any more relevant than Copeland or Rogers.

D. Potter

The device disclosed in Potter was designed for the special needs associated with enzyme reactions, hybridization and melting of nucleic acids, and thermal cycling of samples for amplification of DNA. These types of laboratory techniques require small amounts of expensive or difficult to obtain samples. As such, one of the primary concerns is reduction of evaporation

while the samples are being heated and cooled. To prevent condensation and evaporation of the sample, the top of the container is covered with a lid that has heating elements (See column 7, lines 6-12.)

Potter discloses an apparatus capable of independently regulating the heating of each sample in a sample container designed for rapid heat transfer to a set temperature. According to FIGS. 1 and 2 of Potter, within the sample plate 10, the sample 11 is in the form of a thin disc of fluid contained in a well 13. Each well 13 is sealed at the top by sealing foil 15 and sticky seal 17 or heat sealed after the samples 11 are placed in them. The base of the well 13 is likewise shielded.

4. Differences Between the Prior Art and Applicants' Claimed Invention

The Examiner is correct that both Copeland and Rogers relate to microscope slide staining and disclose a liquid dispenser and a means for automating the dispensing of reagents onto a microscope slide. However, as described more fully below, this statement is incorrect with regard to both Heidt and Kerr. Additionally, the Examiner has oversimplified Applicants' invention by stating that the four primary references "are directed to the automated processing of slides encompassing all of the claimed elements except for an individual sensor beneath each slide to control each slides [sic] temperature independently."

None of the primary references, Heidt, Copeland, Kerr or Rogers, discloses a platform supporting a plurality of microscope slides, the platform having plural heated surface areas, each heated by an electric heater thereunder, each heated surface area being adapted to be in contact with and underlie a microscope slide bearing a biological sample and having plural temperature sensors on the platform for sensing temperature of respective heated surface areas as claimed in the present application.

As described above, for most embodiments, the primary references rely on convective heating to heat the slides rather than conductive heating enabled by the claimed plural underlying heated surface areas.

Rogers does illustrate, in one embodiment represented by Figures 7 and 8, the conductive approach to heating. However, in the embodiment disclosed in Figures 7 and 8, slides are slowly advanced across a stationary platen 94. There is no suggestion of plural heated surface areas,

each heated by an electric heater and underlying a microscope slide with temperature sensed by a respective temperature sensor. Furthermore, in this embodiment, Rogers teaches that "[t]he various reactants are applied to the specimen-containing surfaces of the slides through orifices in the platen." Because the slides rest upon the platen, Rogers does not teach a liquid dispenser that dispenses liquid reagent onto the slide bearing the biological sample, said liquid dispenser being located above the platform.

Additionally, as discussed below, neither Heidt nor Kerr relates to microscope slide staining. Instead, each is directed to an apparatus used in the analysis of blood in which a drop of serum is dispensed onto a chemically reactive analyte slide.

Potter also does not relate to microscope slide staining and fails to teach a dispensing assembly that includes a platform and a liquid dispenser, wherein the platform can support a plurality of microscope slides. In fact, Potter does not disclose a liquid dispenser. As such, Potter fails to disclose a dispensing assembly with random access slide staining capability.

5. A combination of Heidt or Kerr with Potter would not result in the claimed invention

Even if one of skill in the art had found been motivated to combine the heating system of Potter with either Heidt or Kerr, the invention as claimed would not result because none of the references are directed to microscope slides bearing a biological sample.

Instead, as described above, Heidt and Kerr are both directed to the analysis of chemical analyte slides upon which a drop of serum is dispensed. The chemical analyte slide of Heidt and Kerr comprises reagent used for chemical analysis of blood.

Potter is directed to analysis of liquid samples in wells rather than tissue samples on slides.

There is no evidence to suggest that one of skill in the art would have found it obvious to modify any of Heidt, Kerr or Potter to accommodate a microscope slide bearing a biological sample.

Additionally, in order to accurately analyze the chemical analyte slides of Heidt or Kerr, the temperature at which each slide is incubated must be the same for all slides. Therefore, the separate heating control of Potter, would be contrary to the reaction conditions required for

analysis of chemical analyte slides. In view of this fact, Heidt and Kerr teach away from any combination with Potter.

6. Without Improper Hindsight Bias, There was no Motivation to Combine the Heating System of Potter with the Apparatus of Heidt, Copeland, Kerr or Rogers

In *KSR*, the Supreme Court relied upon two references, both directed to the same field of endeavor as the claimed invention (accelerator pedals) for its determination of obviousness. The fact that both references were directed to the same field of endeavor as the invention made it much more likely that one of skill in the art would have been motivated to combine the references to gain the advantages of both references.

In this case, on the other hand, the Examiner has cited five different references to support his rejection under 35 U.S.C. §103(a). Of those references, only two, Copeland and Rogers, actually relate to the same field of endeavor as Applicants' claimed invention (microscope slide staining). Heidt and Kerr, discussed *supra*, relate to the field of chemical analysis of blood, while Potter, also discussed *supra*, relates to the fields of enzyme reactions, hybridization and melting of nucleic acids, and thermal cycling of samples for amplification of DNA. Because Potter does not relate to the same field of endeavor, there is less likelihood that one of skill in the art would have been motivated to combine its heating system with any of Heidt, Copeland, Kerr or Rogers.

In fact, Applicants have provided ample evidence that, at the time of the invention, there was no perceived need for a microscope slide stainer having random access dispensing assembly comprising plural heated surface areas, each heated by an electric heater thereunder and having plural temperature sensors for sensing temperature of respective surface areas. Without motivation to combine Potter with any of the primary references, the Examiner has based the 103(a) rejection upon improper hindsight bias to identify portions of the invention from a different field of endeavor.

Histochemical staining of biological samples as in Copeland and Rogers requires sequential application of at least one stain and a wash solution to remove the excess stain. Actually, almost all histochemical processes require multiple stains or reagents, interspersed by washes. A system in which the samples are sealed off from the outside environment in sample

wells, like the apparatus described in Potter, would not be compatible with the needs associated with automated microscope slide staining protocols. Thus, one of skill in the art would not look to Potter when designing a dispensing assembly wherein liquid reagents are dispensed onto the microscope slides from above as claimed in the present invention.

Furthermore, Potter describes a heating block adapted for specially designed microplate wells containing liquid samples. Potter does not describe staining of tissue sections on microscope slides. Potter makes no mention of slides, histochemistry, or tissue samples. Instead, Potter relates to analyzing a liquid sample 11 (Column 3, lines 55-56). The samples are in wells 13, such as in a microplate format (Column 3, lines 61-63; Column 7, lines 5, 11, and 18-21; Fig. 1). Unlike a transparent, rigid, low thermal conductivity microscope slide, Potter teaches that the sample is placed on foil 12 for its high thermal conductivity. (Column 3, lines 56-58). Further, metal foils are neither transparent nor rigid. These differences emphasize the fact that Potter discloses an apparatus for uses that are quite different from histochemical staining of tissue samples. Therefore, the reasons stated in the specification of Potter (Column 1, line 9 through Column 2, line 29) regarding the desirability for varying the temperature of samples must be viewed in the context of liquid biological samples, and do not translate to slide staining. The teachings of Potter do not provide motivation for one of ordinary skill in the art of slide staining to combine Potter with the cited primary art references.

On the other hand, within the field of slide staining described above in Section B, there was no apparent reason for conductive heating using a platform supporting a plurality of microscope slides, the platform having plural heated surface areas, each heated by an electric heater thereunder, each heated surface area being adapted to be in contact with and underlie a microscope slide bearing a biological sample and the platform having plural temperature sensors for sensing temperature of respective heated surface areas.

In fact, the device disclosed in Potter would be unsuitable for use with slide staining, and the apparatuses described in Heidt, Copeland, Kerr and Rogers are incompatible with the laboratory techniques involving liquid samples as envisioned by Potter.

Additionally, the heating control of Potter would be contrary to the reaction conditions required by the chemical analyte slides described in Heidt and Kerr. Both Heidt and Kerr disclose devices for use in clinical chemistry for measuring the concentrations of various

chemicals in blood. Analysis of the chemical analyte slides does not require the heating control as disclosed in Potter. In fact, in order to accurately analyze such slides, the temperature at which each slide is incubated must be the same for all slides.

For these reasons, the heating control taught by Potter is not meaningful in the context of slide staining or in the context of analysis of chemical analyte slides. At the time of the invention, the benefits of plural heating surfaces in a PCR cycler as proposed by Potter were not recognized as having any meaningful benefits in the field of slide staining.

Additionally, the inclusion of plural heated surface areas, each heated by an electric heater thereunder in a random access dispensing system imposes significantly greater technical challenges and expense. As such, one of ordinary skill in the art would need a compelling reason to undertake the technological and economic challenges associated with such modifications. As described above, in 1994 (the year of filing), no such compelling need existed.

7. Contrary to the Expectations of Those of Skill in the Art, Applicants' Invention Enabled Automation of Special Stains

One of ordinary skill in the art at the time of the invention did not believe that special stain techniques could be automated. As evidence of this belief, Applicants have attached as Exhibit D, an article published in 2000 by Ventana Medical Systems, more than five years after the priority date of the this Application. In this article, Ventana Medical Systems, the leader in the field, discusses the "art" of special stain techniques (page 120) as well as the difficulties associated with automating special stain processing (pages 117-118). The Ventana proposal was to adapt all special stains to a single processing temperature, relying on a dry forced air heater capable of heating all samples to 60°C (pages 118-119).

One of the main advantages of the present invention is that it enabled automation of special stains. Prior to automation, special stain techniques often required judgments on the part of the technician, such as color analysis. Namely, the technician dipped the slide in a chemical or dye until the tissue elements acquired a certain specified color, as determined visually. Because such techniques rely heavily on the skills of the technician, and are considered an art, they had not been considered appropriate for automatic processing.

As the system was further described with independent temperature control (later application number 09/032,676, now U.S. Patent No.: 6,183,693), Applicants recognized that even special stain processes could be performed in an automated system by strictly controlling variables such as reagent concentration (disposing of the reagent after use instead of re-using it, which was previously the most common practice), temperature and incubation time. Contrary to beliefs of those skilled in the art, precise control could take the place of the art required prior to the present invention and such precise control was enabled by the claimed invention.

Accordingly, it is submitted that none of the references, alone or in combination, disclose plural heated surface areas, each heated by an electric heater thereunder, wherein each heated surface area is adapted to be in contact with and underlie a microscope slide bearing a biological sample. Thus, the rejection under 35 U.S.C. § 103(a) is respectfully traversed and reconsideration is requested.

Information Disclosure Statement

An Information Disclosure Statement (IDS) is being filed concurrently herewith. As noted in the Supplemental Information Disclosure Statement filed on January 20, 2006, the parent of the present application is involved in an infringement action. In that proceeding, defendant has alleged that inequitable conduct was committed during the prosecution of the parent application. Although these allegations are baseless and the arguments presented by the defendant are not believed to be material, Applicants are providing a redacted version of the filing made by the defendants. The unredacted version is subject to a protective order.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

By _____
James M. Smith
Registration No. 28,043
Telephone: (978) 341-0036
Facsimile: (978) 341-0136

Concord, MA 01742-9133
Date: 10/21/7

Advances in Anatomic Pathology
Vol. 7, No. 2, pp. 110–122

An Update on "Special Stain" Histochemistry with Emphasis on Automation

*Thomas Grogan, †Kurt Reinhardt, *Marina Jaramillo, and *Darlene Lee

*Department of Pathology, University of Arizona, Tucson, Arizona, U.S.A., and †Ventana Medical Systems, Tucson, Arizona, U.S.A.

Summary: For nearly 100 years, pathologists have utilized "special histochemical stains" to assist in tissue-based diagnosis. As illustrated in Figures 1 and 2, histochemical stains have been used to identify infectious microorganisms (e.g., *Mycobacterium tuberculosis* with acid-fast bacillus (AFB) stain), to detail inflammatory stromal or structural alterations (e.g., fibrosis in liver cirrhosis with Masson trichrome), to identify microanatomic sites of disease (e.g., basement membrane in glomerulonephritis with Jones methenamine silver), to identify abnormal chemical deposits (e.g., iron in hemochromatosis with Prussian blue stain), or abnormal immune deposits (e.g., amyloid via Congo red stain). The current surgical pathology laboratory may employ a repertoire of 20 to 25 "special stains" to ensure the full diagnostic complement.

While the diagnostic repertoire and the biochemical recipes for the stains are now a well-established, codified part of surgical pathology, there is an ever-moving, leading edge of new developments including new reagents, applications, and methods. This review seeks to update the reader on some of the new applications including both new reagents and methods. Particular emphasis will be placed on the recent technologic advance of automating special stains in kinetic-mode (1–4). The authors consider in turn: 1. In brief, the "news" (recent literature review) of new staining applications; 2. In greater detail, two new applications for detection of *Microsporidia* and *Helicobacer pylori*; 3. The new technologic advancement of kinetic mode automation of special stains. **Key Words:** Special stains—Histochemistry—Automation—Kinetic mode

LITERATURE REVIEW OF NEW STAINING APPLICATIONS

Mucin Stains

Mucicarmine stains are routinely used in histologic laboratories to demonstrate the presence of mucin in tissue sections. This special stain provides useful information when used to supplement routine hematoxylin- and eosin-stained tissue sections, to address such diagnostic dilemmas as determining the gastrointestinal mucosa origin of a poorly differentiated carcinoma, or distinguishing Paget disease from melanoma, or muciphages from signet-ring carcinoma (5). New applications for mucin stains include:

1. applying a mucin stain to frozen sections,
2. investigating Hirschsprung's disease,
3. studying the origin of gastric carcinoma.

Frozen Section Mucin Stain

Routine mucicarmine stains are usually applied to paraffin-embedded, formalin-fixed tissue. On occasion, diagnostic difficulty may be resolved by the demonstration of mucopolysaccharides in a frozen section. Surgical pathologists at Hahnemann University Hospital in Philadelphia, PA, addressed this problem by modifying a standard mucicarmine stain, resulting in a 3-minute staining method that can be used on frozen sections (5). Performing this rapid method in addition to the normal hema-

Address correspondence and reprint requests to Thomas M. Grogan, M.D., University of Arizona, Health Sciences Center, Department of Pathology, Room 5212, 1501 N. Campbell Avenue, Tucson, AZ 85724.

toxylin- and eosin-stained frozen section slide did not significantly prolong time turn-around. The modified mucicarmine method involves staining the frozen section slide first with hematoxylin, followed by application of concentrated mucicarmine solution that has been heated in a microwave for 30 seconds, and then counterstained with light green. This technique maintained nuclear detail (nuclei stained blue with the hematoxylin), while demonstrating the presence of mucopolysaccharides in epithelial and connective tissue cells (mucins stained deep rose, connective tissue stained faint green). The major utility of this stain is to assess margins for malignant, mucin-containing cells that may be difficult to definitively distinguish on hematoxylin- and eosin-stained frozen sections (5).

Mucin in Gastric Carcinoma

Histochemical analysis of mucin has also been used to phenotype gastric carcinomas. A recently published study subjected 56 intramucosal differentiated adenocarcinomas of the stomach to a battery of different mucin stains to determine the phenotypic expression of mucin produced by the carcinoma. The tumors that stained positively with a high-iron diamine-alcian blue stain, which detects sialomucin and sulfomucin, were deemed to express an intestinal phenotype. Neoplasms that stained positively with a concanavalin A-horseradish peroxidase technique, which demonstrates class III mucins, indicated a gastric phenotype. The presence of gastric foveolar-type mucin was indicated by a positive galactose oxidase Schiff reaction. The study concluded that minute gastric carcinomas tend to express a gastric phenotype, and are histogenetically derived from the gastric glands. As the diameter of the tumor increased, gastric phenotype decreased, accompanied by a concommitant increase in mixed gastric-intestinal phenotypic expression or intestinal phenotypic expression by the tumor cells; this switch in phenotypes was correlated with the presence of intestinal metaplasia in the mucosa surrounding the gastric carcinoma (6).

Mucin in Hirschsprung's Disease

Patients with Hirschsprung's disease produce decreased amounts of mucin in their colonic mucosa, resulting in a deficient defensive barrier. Eight different mucin genes have been identified by molecular techniques. Investigators in England have used a mild periodic acid-Schiff (mPAS) stain, in conjunction with mRNA in situ hybridiztion techniques, to identify the mucin genes expressed in the colonic mucosa of Hirschsprung's patients and normal controls. The study concluded that the mucin genes expressed in the two test populations did not differ significantly, and that the mechanism controlling mucin secretion by the colonic mucosa is intact (7).

Special Stains Versus Immunohistochemistry

The latter two studies illustrate how mucin special stains have a research value. Special stains are of particular value in evaluation of mucins, mucopolysaccharides, and complex carbohydrates. This contrasts with immunohistochemistry (IHC), which provides protein markers. This difference in detection reflects the fact that IHC monoclonal antibodies are typically produced in mice in response to human protein antigens (immunogens,) where substantial species protein nonhomology exists. In contrast, carbohydrate/mucin complexes are typically largely homologous and shared among vertebrates producing little immunogenic effect, making production of a monoclonal antibody very difficult. For this reason biochemical-based special stains are favored as probes for sugars and IHC as probes for proteins.

Spectral Image Analysis and Special Stains

Another application of special stains is as an adjunct for other histopathologic methods. Spectrally resolved imaging is considered to be an objective tool for characterization and comparison between different cell types. It uses a light microscope to perform a multipixel spectroscopic analysis of the colors formed by chromatin-stain complexes. In 1998, a study by Rothmann et al. (8) applied this technique to distinguishing the nucleolar differences between normal human hepatocytes and hepatocellular carcinoma. In this study, formalin-fixed, paraffin-embedded liver tissue was stained with hematoxylin and eosin, Romanowsky–Giemsa, periodic acid-Schiff (PAS), or Masson's trichrome stains. At least 10 cells from each stained slide were analyzed by multipixel spectral analysis, and the transmitted light spectra of the three nuclear domains (nucleolus, chromatin, and interchromatin domain) were determined for each staining method. In hepatocytes stained with H&E, Romanowsky–Giemsa, and PAS, the transmitted light spectra for the chromatin and nucleolus were similar, while the spectra of the interchromatin domain was different. Hepatocytes stained with Masson's trichrome revealed a distinctly different nucleolar spectrum, distinguishing it clearly from the chromatin. The authors concluded that this method could be used to demonstrate nuclear differentiation in paraffin-embedded tissues, and provide an objective histologic distinction between normal and pathologic processes (8). This improved imaging capability enhanced by special stains may be especially useful in generating newly described "nuclear signatures" in de-

veloping neoplasia (9). These machine-generated "nuclear signatures" now detail the initial morphologic steps of neoplasia (e.g., skin cancer), and are also providing intermediate markers for cancer chemoprevention (e.g., green tea) (9).

Silver-Based Stains: Steiner Stain

The modified Steiner stain is a silver-based stain commonly used for the identification of bacteria in formalin-fixed, paraffin-embedded tissues, specifically *Helicobacter pylori* in gastric biopsies, and *Treponemas* in various sites. Recently, Saiz et al. (10) observed that the modified silver stain also stains the viral inclusions of cytomegalovirus-infected cells with a sensitivity that closely approaches that of immunohistochemistry (93%). Whether the modified Steiner stain will become a useful screening tool for the detection of cytomegalovirus infection remains to be proven. Issues related to specificity have to be addressed. In addition, the need for the use of uranyl nitrate, a toxic radioactive substance, is an added disadvantage that has to be taken into consideration in the final analysis of this stain.

Silver-Based Stains: Myelin Stain

McNally et al. (11) recently described a new method for the intense staining of myelin. This technique involves the immersion of frozen or vibratome tissue sections in 4% normal horse serum, followed by a diaminobenzidine (DAB) reaction that causes the myelin sheaths to become light brown. The silver enhancement technique described by Gorcs (12) follows, resulting in the specific staining of myelin sheaths. After this intensification procedure, the myelin stains an intense black color, and even individual fibers can be easily visualized. By electron microscopy, deposition of metallic particles is seen only within myelin sheaths. Interestingly, these particles are not specifically related to any single component of the sheaths. The principle underlying this technique is not completely understood. It has been speculated that there is an oxidizing agent in the horse serum that binds to myelin and its derivatives, causing the release of oxygen and the subsequent polymerization and deposition of DAB, which can be visualized after intensification. Because of its specificity and the intense stain achieved, this technique can become a useful tool for the examination of the distribution of nerve fibers in gray matter, particularly in the research setting. This technique in embedded tissue is less consistent, with uneven staining, limiting its usefulness.

Periodic Acid-Silver Methenamine Stain

Periodic acid-silver methenamine stain (PAM) is a silver impregnation stain commonly used for the delineation of basement membranes. In a recent study by Katoh et al. (13), PAM stain was successfully used to highlight stromal deposits of basement membrane material in four cases of hyalinizing trabecular adenoma of the thyroid. This eosinophilic hyaline substance demonstrated strong reactivity for both type IV collagen and laminin, consistent with the hypothesis that it represents basement membrane material. The pattern of deposition, mesh-like, fir-branching with a flame-like appearance, was most clearly seen with PAM stain. Electron microscopic evaluation of the samples was also performed, revealing characteristic ultrastructural features. The authors postulated that these findings, including electron microscopy, immunohistochemistry, and PAM impregnation, might be useful in the differentiation of trabecular adenoma of the thyroid from other more aggressive thyroid tumors.

Silver-Based Stains: Light Insensivity

Histologic stains relying on silver reduction have been in use almost as long as photography itself. Traditionally, these stains have been employed to visualize tissue structures such as reticulin fibers, but in a blending of old and new, silver stains are being used to amplify markers in immunohistochemistry and in situ hybridization. Current work centers on developing silver formulations, which are light insensitive—long the principle difficulty in these types of applications. Interestingly, automation may provide another avenue for addressing this problem, as an automated system may perform its actions completely in the dark, an option not available to human technicians (14).

NEW STAINING APPLICATIONS DESCRIBED IN DETAIL

Detection of Microsporidia in Tissue Specimens

In 1959, a new etiologic agent (Microsporidia) that causes diarrhea in humans was identified. Prior to the AIDS pandemic, only eight cases of diarrhea due to this microorganism were recorded in the literature. Microsporidia is currently a leading cause of chronic diarrheal illness in HIV-positive patients, with an incidence of 5%–30% of the AIDS population (15). In 1994, the first case of microsporidial disease in *nonimmunocompromised* patients was reported, and it has since been recognized as a cause of travelers' diarrhea (16). Recently, microsporidia were identified as a cause of infection in transplant patients (16,17). As the population of immunocompromised patients grows, the need for efficient, cost-effective and sensitive screening tools for etiologic agents of opportunistic infections increases.

Microsporidia are primitive eukaryotes that are obligate intracellular parasites. They belong to the *P. micros-*

pora, which is composed of more than 1000 species; however, most species infect insects, fish, and birds. Twelve species of microsporidia are known to infect humans, and many of these also infect insects and/or animals. These organisms are responsible for infections of the small intestine and biliary system (*Enterocytozoon bieneusi*); disseminated infections to the kidney, liver, sinuses, and brain (*Encephalitozoon sp.*, *Microsporidium sp.*); corneal infections (*Nosema sp.*, *Vittaforma corneae*); and myositis (*Trachipleistophora sp.*, *Pleistophora sp.*). The mode of transmission of these pathogens is unknown, but thought to be either via the fecal-oral or urinary-oral routes; transplacental transmission has been documented in carnivores, but not in humans. Microsporidia have been isolated from surface water supplies in France, as well as from raw sewage and ground water supplies in the U.S. A retrospective study in France linked a presumed water-borne outbreak in AIDS patients to a common water source in Lyon (16). Although a carrier state has not been documented in humans, it is felt that it likely exists, as other animals (rabbits and rodents) have chronic subclinical infections, and recent studies report the seroprevalence of microsporidial infection as 3%–8% of Dutch blood donors and 5% of pregnant French women (16,17).

The infectious stage of the organism is the oval-shaped spore, which remains viable in the environment for more than 1 year (16). The spore is quite characteristic, consisting of an outer electron-dense glycoprotein coat, the exospore, which encloses an inner endospore composed of electron lucent chitin. Within the endospore is a unique polar filament, which injects the spore contents into the host cell under suitable conditions. Once inside the host cell, the organism undergoes successive divisions, generating more spores until the host cell eventually ruptures, releasing the spores into the surrounding milieu, where they can infect adjacent cells.

The most common clinical manifestation of microsporidial infection in humans is diarrhea, which tends to be self-limited in immunocompetent patients, but is usually chronic in AIDS patients. This finding has been correlated to a low CD4+ T cell count (≤ 100 CD4+ T cell/ul of blood) and a high HIV viral load; thus, antiretroviral therapy has been shown to reduce microsporidia infection by virtue of decreasing the HIV load and increasing the CD4+ T cell count (16–18). The most effective treatment for microsporidial infections is Albendazole (SmithKline Beecham, Philadelphia, PA), a microtubule inhibitor, but it is only variably effective against the most common infective organism, *E. bieneusi*. Because of this, speciation of the infectious microsporidia may help direct therapy.

The gold standard for the diagnosis of microsporidiosis is transmission electron microscopy; however, this method is time-consuming, expensive, may not be widely available, and is not suitable as a screening method (15,17). Other commonly used methods include routine hematoxylin and eosin staining of tissue sections, Gram stain, Giemsa stain, Warthin–Starry stain, and a modified trichrome stain. Several studies have been published comparing the sensitivity of different staining methods in fluids, stool and tissue sections (15,19,20). The most recent (1998) comparison by Lamps et al., compares polarization, special stains, and molecular techniques in the screening of tissue sections for microsporidia (15). The most sensitive special stain in this study was the modified trichrome stain, which detected microsporidia in all cases that were positive by any stain in the battery, including hematoxylin and eosin, Warthin–Starry, Brown–Brenn, methenamine silver, and acid-fast stains (15).

The modified trichrome stain proposed in the study by Lamps et al. (15) is based on the work of Weber, who developed a trichrome stain for screening stool specimens for microsporidia. The Weber-modified trichrome stain stained microsporidial spores red; however, background fecal material and yeast cells also stained red. In addition, the Weber stain lacked a background color, and the screening of large numbers of slides was tedious and time-consuming (21). In 1993, Ryan et al. (21) modified the Weber stain by decreasing the phosphotungstic acid content of the stain and using aniline blue as a counterstain. Additional manipulation of the temperature of the stain and the staining time resulted in organisms that stained a more vibrant color against a sharper, clearer background, as well as allowing faster specimen processing times and a decreased time to diagnosis (22). As this modified trichrome stain was initially developed for use with fecal specimens, researchers at the University of Washington, Seattle, WA, adjusted the concentrations of the reagents and the staining times in order to adapt the aniline blue modified trichrome stain for use with paraffin-embedded, formalin-fixed tissue sections. The end result is an inexpensive, reproducible, easy-to-perform technique that stains the microsporidial spores an intense red against a background of pale blue, making this method an ideal screening tool (15).

Trichrome stains are a category of procedures that use different dyes to demonstrate different elements of the specimen. In tissue sections, dyes of differing molecular size can be used to selectively stain muscle, collagen fibers, fibrin, and erythrocytes. Generally, the smallest dye molecules penetrate the less porous tissues, and if a

larger dye molecule is able to penetrate the tissue, it does so at the expense of the smaller dye molecule (23). To prevent mixing of colors, dyes are used sequentially, in order of increasing molecular size (24). The addition of phosphotungstic acid greatly reduces the staining of all tissue components (except collagen fibers) with aniline blue, as the phosphotungstic acid acts as a "colorless dye," selectively replacing anionic dyes such as aniline blue. Phosphotungstic acid is less efficient at replacing smaller dye molecules (23,24). In the aniline blue modified trichrome stain, the smaller molecular-sized dye (Chromotrope 2R) is combined with the larger molecular-sized dye (aniline blue) and phosphotungstic acid to prepare a single reagent. This single step combination most likely allows the glycoprotein exospore of the microsporidia to bind the Chromotrope 2R dye tightly while the phosphotungstic acid competes with the aniline blue for the binding sites of the background tissue components, resulting in decreased staining of the background elements. The aniline blue modified trichrome stain appears to be a highly sensitive stain specific for microsporidia (15).

Molecular techniques for detection of microsporidia in clinical specimens are available. These methods include immunofluorescence assays (IFA) and polymerase chain reaction (PCR) assays. In early IFA studies, nonspecific staining of other organisms present in stool specimens limited their usefulness as a screening tool (19), but with the development of species-specific antibodies to microsporidia, there may be a confirmatory role for this assay, with the added benefit of speciating the organism. Polymerase chain reaction methods are more expensive than special stains, but can be useful for both diagnosis and speciation. However, PCR methods appear to be dependent on organism load, sampling, and focality of infection (15). Due to such restrictions, use of this method for screening purposes is impractical at this time; however, it plays a valuable role as a confirmatory test and to identify organisms to the species level, which may be important in directing therapy.

Preventive measures against microsporidial infection are difficult to define, given the limited knowledge regarding the organism's mode of transmission, environmental reservoir, infectious dose, carrier status, and true incidence of infection in the general and immunocompromised population. At this time, there is no information regarding the risk of infection to caregivers and others in contact with immunocompromised patients who harbor a microsporidial infection, which suggests that there is a possibility of nosocomial outbreaks. The presence of the organism in U.S. water supplies implies that waterborne outbreaks are highly probable. All these issues emphasize the importance of developing a special stain that serves as a sensitive, rapid, and cost-effective screening method for detecting microsporidia.

Histochemical Stains for *Helicobacter Pylori*

The discovery of the role of *H. pylori* in the pathogenesis of gastroduodenal disease, including chronic active gastritis, ulcer peptic disease, gastric carcinoma, and gastric lymphoma of mucosa-associated lymphoid tissue (MALT)-type has created a great interest in this organism and has led to the development of several new special stains for the detection of bacteria on histologic sections (25,26). *Helicobacter pylori* infection is a worldwide problem with a prevalence that increases progressively with age, reaching 50% or more by age 60 (27). It has been demonstrated that complete eradication of the organism from the gastric mucosa by triple antibiotic therapy leads to resolution of the inflammatory process, and even regression of the some documented cases of mucosa-associated lymphomas (28,29).

There are a number of methods available to detect the presence of *H. pylori*. These include culture, serologic tests, urea breath test, and histology. Culture is difficult, given the slow-growing and fastidious nature of this organism. Serologic tests, although very useful as epidemiologic tools, are unreliable to assess response to therapy, since they remain positive for long periods of time after the conclusion of the treatment. The urea breath test is highly specific and sensitive, but it does not allow morphologic evaluation of the gastric mucosa for the presence of *H. pylori*-associated diseases. For these reasons, histologic assessment of multiple gastric biopsy specimens continues to be considered the "gold standard" in the evaluation of *H. pylori* status. Identification of *H. pylori* on H&E stains can be time-consuming and leads to inconsistent results (see Figure 1), particularly when dealing with low numbers of bacteria, as usually occurs in cases of persistent infection after treatment. For this reason, many pathologists use a second stain to facilitate the visualization of the organism. The special stains traditionally used for this purpose include Warthin–Starry stain, Steiner stain, Giemsa stain, and Diff-Quik stain (Figure 1). The Warthin–Starry stain is technically cumbersome and its quality is highly dependent on the histotechnologist's experience. Giemsa and Diff-Quik stains, although sensitive, do not allow an accurate assessment of the underlying tissue morphology (30). In the last few years, several new improved techniques for the detection of *H. pylori* in tissue samples have been developed, some of which will be briefly reviewed in this article.

The Genta stain, developed in 1994, is a silver-based

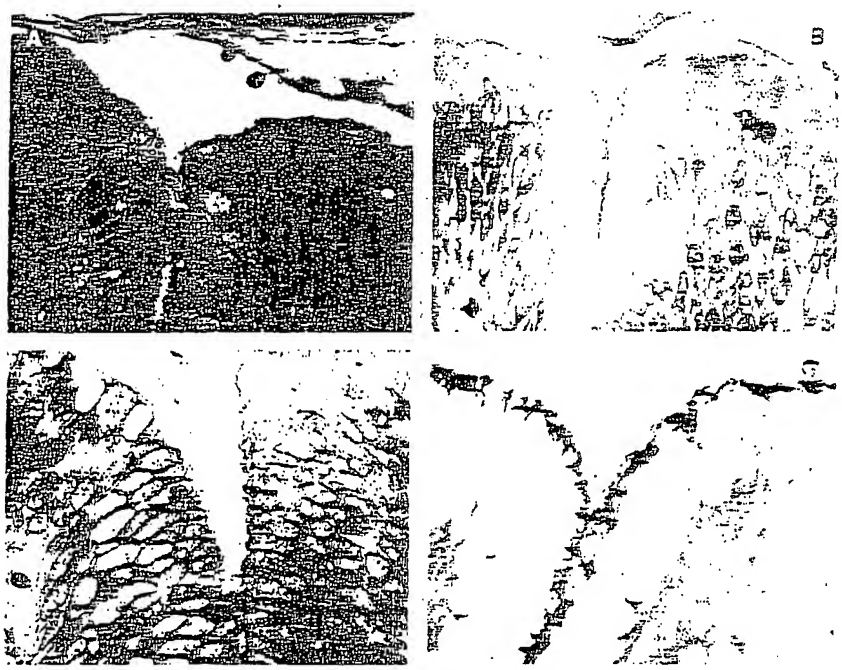

FIG. 1. Detection of *Helicobacter pylori*. The comparative features of *H. pylori* staining are shown in a gastric biopsy stained with hematoxylin and eosin (A), alcian yellow (B), giemsa stain (C), and with a monoclonal antibody to *H. pylori* (D). As demonstrated, the surface mucosa-associated curvilinear organism is readily seen with special stains of alcian yellow and giemsa, and by immunohistochemistry (IHC). This contrasts with the hematoxylin and eosin image where diagnosis is less certain. The ready stain with alcian yellow and giemsa argue for utility as a screening tool: while IHC, which may be more specific but less sensitive, could be used for diagnosis confirmation.

stain that allows simultaneous visualization of the bacteria and of the histologic features of the tissue under evaluation, including assessment for intestinal metaplasia (30). From a technical point of view, the Genta stain is a hybrid that combines a Steiner stain, H&E stain, and an alcian blue stain at pH 2.5. The Genta stain provides a pronounced contrast between the dark brown bacteria and the red-staining background, greatly facilitating the identification of the organism. In a recent study, El-Zimaity et al. (31) compared the sensitivity and specificity of this stain to other two commonly used techniques, Diff-Quik and Giemsa, in the detection of *H. pylori* after therapy. Their results demonstrated that Diff-Quik and Genta stains had comparable sensitivities (96% and 94%, respectively) and similar specificities (99% and 97%, respectively). Giemsa stain, on the other hand, has a comparable specificity (96%) but a somewhat lower sensitivity (86%). The positive predictive value of

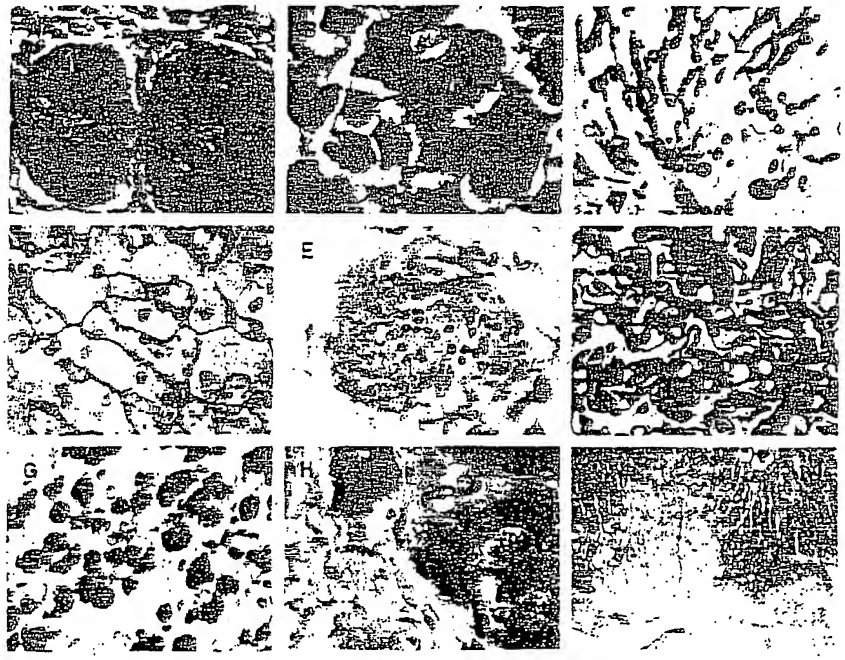

FIG. 2. Demonstration of kinetic mode automated special stains. This composite shows nine assays run and completed simultaneously in kinetic mode within 1 hour by one histotech in a "walk away" mode. The assays all shown at 1000× magnification include: (A) Mucicarmine, colonic mucosa; (B) iron stain, liver hemochromatosis; (C) Grocotts methenamine silver (GMS) stain, aspergillosis of lung; (D) Reticulin stain, liver; (E) GMS stain, *Pneumocystis carinii*; (F) Jones methenamine, renal glomerulus; (G) acid fast bacillus (AFB) stain, *Mycobacterium avium intracellulare*, spleen; (H) Trichrome stain, liver with cirrhosis; (I) Alcian yellow stain, *Helicobacter pylori* on gastric mucosa.

these three stains was virtually identical, ranging from 97% to 99%. According to these authors the time required to assess the *H. pylori* status with the Genta stain is significantly shorter, thanks to the striking contrast between the bacteria and the background. Interestingly, this study also showed that Diff-Quik stain combined with a routine H&E provides accuracy similar to that of the Genta stain alone. A prospective study by Laine et al. (32) compared H&E, Giemsa, and Genta stains in the detection of *H. pylori*. They found that the sensitivities of H&E and these special stains are comparable at about 90%, but H&E has a specificity that is significantly lower than that of the special stains (89% versus 98%). In spite of the excellent quality of the Genta stain, there are some significant disadvantages: it is relatively costly, technically complex, and time-consuming, taking between 1 and 2 hours to complete. In addition, it requires uranyl nitrate, a toxic and radioactive compound, whose use is prohibited in many European countries (31,32).

The Sayeed stain, which combines periodic acid-Schiff, Coleman's Fuelgen solution, Mayer's hematoxylin, and methylene blue, is another of the recently developed special stains for the detection of *H. pylori* in gastric biopsies (33). The organisms stain bright blue and can be easily visualized against a contrasting background of magenta-staining mucin. Similar to the Genta stain this technique has the additional advantage of allowing simultaneous evaluation of the gastric morphology, including detection of intestinal metaplasia, with the use of a single stain. According to a study by Cohen et al. (33), the Sayeed stain has a diagnostic yield for *H. pylori* that is similar to that of H&E and Warthin–Starry stains combined. In addition, this stain is simple to perform and takes approximately 9 minutes to complete, which is significantly shorter than the time required to prepare the Genta stain.

A third recently described stain is the Alcian yellow-toluidine blue (Leung) stain (see Figure 1) (34). According to its developers, this technique has several advantages: it has a low cost, is simple to perform, and takes a short time to complete. In addition, since it does not have the usual problems associated with silver precipitation, it is easily performed by less experienced histotechnologists. With this technique, the blue-staining organism is easily identified against a background of contrasting yellow-staining mucus (see Figure 1). The presence of intestinal metaplasia can also be accurately evaluated with the Leung stain. The Leung stain compares favorably to other more traditional techniques, including Giemsa and the modified Steiner stain. An additional advantage of this technique is the identification of two other gastrointestinal pathogens, *Cryptosporidium* and *Giardia lamblia* (34).

The new *H. pylori* silver stain (HpSS), developed by Dogiioni in Italy, combines a new simple silver stain method with the classic H&E stain, allowing the simultaneous identification of *H. pylori* and the assessment of the gastric morphology (35). This new *H. pylori* silver stain accurately highlights the distinctive morphology of this bacterium. Doglioni and his group found that its sensitivity is comparable to that of immunohistochemistry (98%) and superior to H&E and modified Giemsa stains. In comparison to the Genta stain, which has similar advantages, the new *H. pylori* silver stain is simpler and uses widely available reagents.

Automation of Special Stains: The Need to Automate

Currently, a highly skilled laboratory technician or histologist performs the repertoire of complex special stains manually. In a hospital setting, the technician frequently has to perform special stains assays "on demand" at any time of the day, typically having to mix reagents from scratch from a wide array of reagents. Based on the complexity of the test, the amount of uninterrupted time available, and skill level of the technician, results may vary widely from day to day, site to site, and tech to tech.

The techniques also pose logistical problems for several reasons: they are time consuming both in preparation and performance, require the maintenance of a large inventory of laboratory reagents, and frequently use solutions that begin to degrade rapidly from the moment of formulation. Moreover, repeated exposure to the dye chemistries and solvents may be detrimental to the safety of the technician, and the relatively large fluid quantities used in manual performance require special disposal.

These issues, combined with a declining pool of skilled laboratory histotechnologists, suggest that this is a field poised for automation. The paragraphs below describe the advent of automated instruments for special stains. These paragraphs further emphasize the theory and technical aspects underlying kinetic mode automation. Some of the key mechanical functions are also illustrated.

Characteristics of Automation

Automation, broadly defined, is the augmentation or replacement of human work by that of a machine (36). The motivations for automation are varied including:

- Using machines to replace humans in work which may be hazardous.

- Eliminating inconsistent quality of results, alleviating boredom and injury in repetitious tasks,
- Reducing processing time and lowering cost (36).

Auspiciously, all these considerations apply when considering the automation of special staining procedures.

Automation also varies widely in degree, ranging from simple single step mechanization, to systems that may perform long and complicated series of tasks. To have a meaningful impact in the laboratory, an automated "special stains" stainer would necessarily mechanize all the steps in which reagents are successively applied to a specimen, without human intervention, and ideally, enhance the process by increasing reaction kinetics through temperature control and mechanical mixing.

Any automation project is subject to the precept that since it replaces human beings who may compensate for varying input conditions, it will require standardization of inputs to achieve standardization of results. In the case of histologic staining, these input parameters include degree and type of fixation, tissue section thickness, and slide type and preparation. Automation has the beneficial effect of driving examination and standardization of these parameters resulting in increased uniformity of result.

Fundamental Considerations

Regarding instrument architecture, the question arises whether the platform should process slides in batches, or singly. Batch processing contrasts with the notion of random access, wherein any inactive processing station may be accessed anytime. An optimal hybrid of these approaches is achieved with "rapid multiprotocol batch processing," whereby different protocols may be executed on a group of slides simultaneously. And if the processing is rapid enough, the distinction between batch mode and continuous access becomes moot, as the system achieves a high degree of availability.

Given the wide variety of sizes of institutions that perform special stains, critical considerations for the instrument design include size and capacity. A smaller, lower capacity instrument may be suitable for a small clinic where bench space and capital budget are severe constraints, while a larger, high throughput system would be required for a reference lab or regional testing center. Once again, optimality may be achieved through a hybrid, in this case a modular approach in which a small instrument may be used in tandem with other modules to implement a scalable architecture until the right balance is achieved.

Finally, there is the issue of the degree to which the user must directly specify the steps the automating device performs (i.e., user programming). A platform that automates the steps performed, but requires the user to specify all of those steps, may alleviate one burden while replacing it with another. Many users may lack either the time or expertise, or both, to develop protocols on their own. In these circumstances, a totally instrument-determined solution or set is desirable. Obviously, the acceptability of this instrument-proscribed recipe requires a robust implementation, able to achieve the desired outcome across a wide variety of conditions. Additionally, flexibility can be achieved by a wide offering from a menu of preprogrammed alternative recipes, allowing the user to meet their tastes and requirements with respect to color saturation, hue, choice of counterstain, etc.

Technical Problems to Solve

Special stains encompass a wide variety of stains, employing many reagents and differing chemical mechanisms. Some stains are progressive—achieving increased staining intensity by progressively increasing exposure time and reagent concentration. Others are regressive, totally saturating the target with pigment and then achieving contrast by subsequent washing or decolorizing. Adding to assay complexity, a great variety of ancillary reactants are employed including mordants, clarifiers, decolorizers, and counterstains. This variety immediately raises an important technical question: can the stains be produced in a single instrument environment, or are separate processing environments required for each? Complicating the answer is the knowledge that certain staining reactions such as the silver precipitation in the Grocotts methenamine silver (GMS) stain require elevated temperature (56–58°C) to proceed, while others are typically, though perhaps not optimally, performed at room temperature when done manually. If it could be determined that all special stains are amenable to a single processing temperature, design and control could be simplified.

A related issue entails the complexity of washing buffers. In most procedures, thorough washing of the target slide is required between each application of reagent. This begs the question: is there a single washing medium that can be employed for all procedures? Once again, discovery of a single bulk reagent for washing across all stains would allow a more elegant and simple automated platform design.

Perhaps the most troubling concern in automation of special staining procedures is the danger of reagent crossover contamination. Clearly, if procedures are proceeding in close proximity, or if common elements touch

the reagents, steps must be taken to avoid even small amounts of the wrong reagent contacting a specimen. While some cross contaminants may be nonreactive or benign, there are scenarios where cross contamination may degrade or even totally inhibit the staining reaction. Two common methods employed by medical devices for delivering fluid to a target are aspiration from a vessel and expiration back to the destination, and direct dispensing from an applicator; both have advantages and disadvantages. Direct dispensing from a valve-controlled applicator may suffer from a lack of precision, caused by "hanging drops," and limited ability to deliver differing volumes. Aspiration systems, on the other hand, must be washed between each use, and depending on how this is accomplished, may be a time-consuming process. To wash rapidly, two things are required: 1) a smooth surface where rinsing prevents eddies and prevents contaminants from hiding, 2) rapid flow to carry away contaminants.

The Automated Solution: Kinetic Mode Special Stain

In a series of experiments conducted by the coauthors and others, it was found by optimizing kinetics through automated mixing, heating, and rinsing that special stains could be performed in kinetic mode more rapidly and reliably than by manual methods. It was found that the kinetic method previously applied to immunohistochemistry and in situ hybridization automation greatly aids special stains automation (1–4). It was also found that the great majority of special stains could be performed in a single reaction chamber with some common bulk buffers, obviating the need to independently and simultaneously control multiple environments. This simplification to a single set of control conditions further ensued reproducibility of results. Furthermore, the kinetics of nearly all special stains reactions benefit from elevated temperature, and at worst are not inhibited by it. These findings informed the design of an automated platform described and illustrated below.

Optimized Kinetics

The key to reproducible special stain results optimizing kinetics is to drive reactions to equilibrium or to the same point each time. With kinetic assays the rate changes with time, making them difficult to control. Nonetheless, achieving equilibrium relates to the following key factors:

1. affinity constant (Ka value),
2. concentration of the reactants,
3. ionic strength and pH of the reaction medium,
4. temperature,
5. degree of mixing,
6. unstirred layer effect on diffusion.

The sum of these effects determines the sensitivity of the staining reaction. Sensitivity is the consequence of the signal-to-noise ratio, which in turn relates to the amount of specific activity relative to nonspecific background staining. Background staining is affected by both chemical (e.g., salt concentration) and physical factors (e.g., washing and agitation). The concentration of the reactants is a pivotal factor. In manual staining, lacking control of heat and mixing, increasing concentration, or lengthening exposure time largely drives diffusion of reactants.

Heating and Mixing

Temperature and mixing are critical factors in driving the reactions. At ambient temperatures, manual staining reactions follow the simple principle of diffusion and are generally performed in the circumstance of reactant excess to maximize signal. With an automated system controlling the reaction environment, both heat and mixing may be fully controlled to ensure optimized kinetics. The combination of heat and mixing is important. Heat alone may drive diffusion through increased Brownian motion, but in tissue-staining the components are static, so that the initial reactants become depleted or impede the subsequent reactants through an effect known as the "unstirred layer effect"(2,3) The addition of simple mixing to heating obviates this effect considerably by agitation of the unstirred layer. This agitation allows fresh reactants to reach the sample surface. To mix the reactants on glass slides, as shown in Figure 3, a series of small air jets, driven by a pneumatic air pump, may be used to evenly disperse the reagents and to achieve desired kinetics.

Regarding heating, a dry forced-air heater may be used

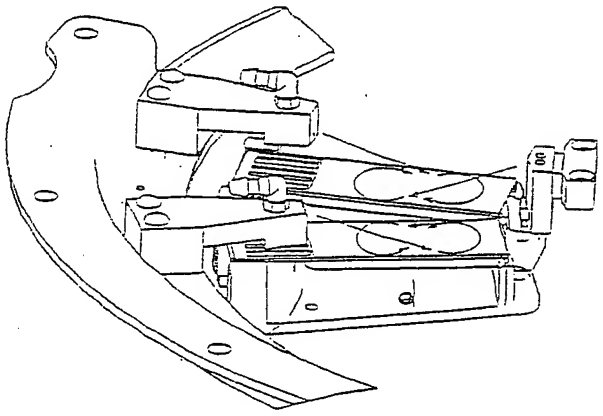

FIG. 3. Air-mixing mechanism.

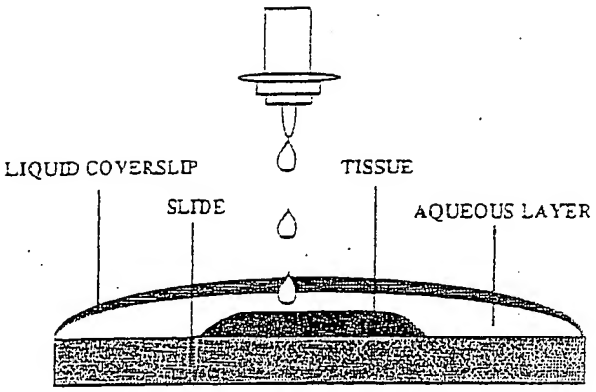

FIG. 4. Liquid cover slips method with reagent dispensed via gravity to tissue.

to drive reaction in the 60°C range for optimal kinetic efficacy. A temperature control system is essential to control the temperature to within ±1.00C. This entails an air thermal detector (thermistor) in the reaction chamber with microprocessor response to control both the forced air (fan) and the heating element (wire coils), resulting in a stable temperature equilibrated environment. To further ensure temperature stability and avoid wide temperature swings with bulk reagent (wash buffer) addition, a heating coil is employed in the bulk reagent bottle to heat fluids before dispensing.

Evaporation Control

Finally, as higher reaction temperatures are sought (60°C), it is critical to ensure that there is no evaporation of the aqueous phase. Evaporation at higher temperature radically affects slide temperature. Indeed, without control of evaporation, virtually all applied heat may be lost via evaporation. Additionally, evaporation leaves a dry slide, ending the staining assay. To control evaporative loss, a liquid coverslip may be used as a sealing, nonreactive protective coat (see Figure 4). Reagents dispensed from above pass by simple gravity through this chemically neutral, evaporative barrier.

Kinetic Washing

To achieve the optimal special stain assay with a minimum of nonspecific background staining (noise), a pivotal factor is tissue washing or rinsing with buffers. Automation offers particular advantages over manual washing. Manual staining methods employ rinsing in between reaction steps in one of two ways: 1) successive dilutions, wherein a section is placed in a bath to dwell for some period and perhaps agitated by hand, or 2) placed under a running tap. Successive dilution has the disadvantage of bleeding reagent into the bath, compromising the effectiveness of the bath, and tap rinse uses copious amounts of water and is also inherently variable in nature. Automation allows a fresh rinse to be directed at each specimen, in discreet amounts sufficient to completely clear the reagents at a tiny fraction of the fluid use. Furthermore, jets (as shown in Figure 5) afford a standing wave effect that serves to remove unbound debris with agitation. The consequence is a minimum of nonspecific background staining and a superior signal to noise ratio. Thus, by vigorous agitation (kinetic washing), automation should produce a superior assay with improved signal to noise ratio. Trichrome stain, as the name implies, uses three successive dyes, separated by differentiating steps. Crisp staining requires complete removal of reagents between steps, lest vibrant red and blue resolve into a muddy purple. Automated kinetic washing should therefore produce a superior assay with less background.

Optimized kinetics ensures first and pseudo-first order kinetics, resulting in a uniform reaction from run-to-run and laboratory-to-laboratory and ensuring highly reproducible results. Additionally and importantly, the ability to run stains in the kinetic mode greatly speeds up the analytic process—greatly improving turnaround time (TAT). Since special stains may be required at any hour to be performed on demand, there is a premium on a rapid, accurate site neutral assay. Some assays (simple progressive reactions) have been run to optimal completion in as little as 4 minutes.

Reagent Dispensing

Given the complex array of chemistry, the wish to avoid hanging drops, and the need to deliver precise volumes, an aspiration system as illustrated in Figure 6 was found most useful. To overcome issues of cross-

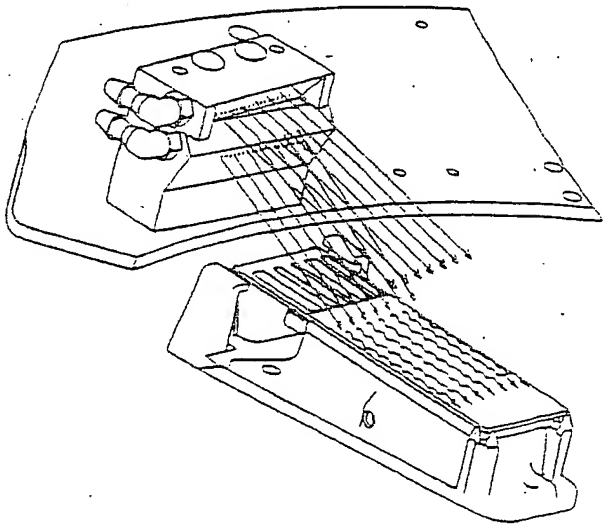

FIG. 5. Discreet jet-washing mechanism creating standing wave effect.

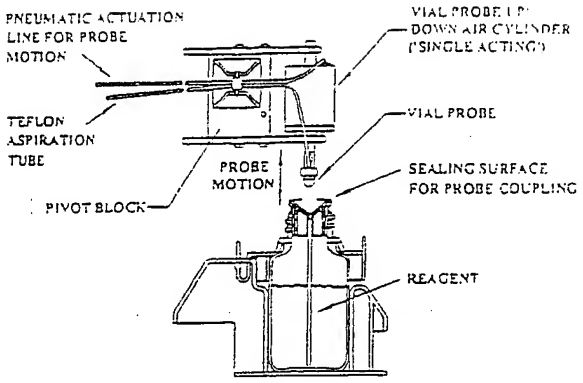

FIG. 6. Reagent aspiration system.

contamination via eddy formation, aspiration was through an inert tubing material (Teflon) with a constant cross-section, which ensures quick, thorough kinetic rinsing (Figure 6).

Examples of Special Stain Automation

The true power of automation lies not in recreating human actions, but through the use of automated devices to perform tasks in ways that a human being cannot. In the case of special stains, this principle manifests itself in several ways: "time of use" mixing of reagents so that solutions are always fresh, accelerated, and reproducible reaction kinetics; the ability to perform multiple complex tasks simultaneously (see Figure 2); and discreet kinetic washing, as described above. Instructive examples of the advantages of automated stains (Grocotts methenamine silver (GMS) staining and Mucicarmine) are described below.

Automated GMS: Improved Reagent Logistics

In the example of Grocotts methenamine-silver nitrate stain, the chromic acid oxidation of polysaccharides begins with immersion in a Coplin jar for 1 hour, followed by subsequent reduction to metallic silver with methenamine silver nitrate solution in a water bath at 56–58°C for 15 minutes. Typical manual performance combines these reagents in solution to create a bath for subsequent sample immersion. The drawback with the manual procedure is that large amounts of reagents are used (often 100 ml or more), and the solution ages very quickly, usually expiring in less than 24 hours, requiring daily reformulation. On an automated platform, these reagents may be stored separately, and then combined *on the slide* at time of assay in very minute amounts (100 to 300 μl). Processing in this manner reduces waste and ensures that solutions used are consistently fresh and does not require daily reformulation.

Automated GMS: Improved Speed and Reproducibility

With kinetic mode automated special stains, reaction kinetics is accelerated in two principle ways: heating and mechanical mixing. The silver reaction in the GMS stain is very temperature sensitive: at temperatures less than 55°C, the reaction nearly ceases, and at temperatures greater than 65°C, can proceed so rapidly that overstaining is nearly inevitable. Moreover, the active reagent is quickly depleted and requires constant stirring to achieve uniform presentation of reactants to the tissue. An automated platform allows not only constant time and temperature control, but can even provide constant mechanical mixing on multiple slides in a way that a human operator could not achieve, thus driving the reaction to equilibrium or to the same point each time ensuring reproducibility.

Improved Simultaneous Performance of Multiple Complex Tasks

The GMS stain has seven distinct assay operations. When done by hand, each of these requires an operator to prepare reagents and then apply them in a carefully controlled manner, sometimes late at night under duress when on hospital call. The inherent variability in a human-centered process requires constant intervention to compensate for inconsistencies. The operator must engage in a routine of "dunk and check" to determine the state of the reaction and determine what should be done next. Obviously, such an approach demands the full attention of a technician, making parallel performance of other staining procedures nearly impossible. In contrast, on an automated platform, numerous procedures can be specified; then through the use of control software be performed simultaneously on the device. For example, one scheme for multiple recipe batch performance is to line the slides up on a rotating carousel, then pass them under regent application and rinsing stations as the carousel rotated at a constant rate. The system may read barcodes to "know" which slide is at which station at any given moment, and can dispense the appropriate reagent. Likewise, the system can "decide" to wash or not wash a specimen depending on the desired incubation or dwell time specified for a step in the operation. Thus, as many recipes can be simultaneously performed as there is space on the carousel. As illustrated in Figure 2, as many as 9–12 assays may be performed simultaneously within 1 hour to facilitate same day surgical pathology sign out.

Site Neutrality Improved by Automation

In their present state, producing special stains can be rightly thought of as much an art as a science: practitio-

ners vary their techniques and formulations to achieve subtle differences in hue, intensity, and even sensitivity and specificity. Broad variability has evolved from site to site, and practitioner to practitioner, until the same stain can appear markedly different from one institution to another or even from different days in the same institution. While the ability to fine tune a stain to achieve some subtle outcome is often rightfully a point of pride for technicians, there are potential pitfalls. While the small nuances of a stain are often a matter of preference and familiarity, they can also be diagnostically significant differentiators. A case in point is the Mucicarmine stain. It is well known that mucin produced in different areas of the gastrointestinal tract (GI) tract respond with different intensities to Mucicarmine stain. The procedure for producing the stain relies on consistency of performance from specimen to specimen to make this distinction apparent. The stain can be overdriven, however, and cause dark mucin staining from almost any specimen. While the richer staining may be more aesthetically appealing, the ability to assess the appropriateness of the mucin staining is compromised. Accordingly, the conclusions drawn in references 5–8 could be altered. A standardized site-neutral automated assay would seem to obviate this uncertainty.

Conclusions

The field of special stains histochemistry is a gold mine of chemical techniques, clinical applications, and sometimes forgotten knowledge. This update recognizes the importance of this field of knowledge and highlights that there is a steady stream of new developments. Besides new clinical applications of old biochemical recipes, there is also a new generation of instruments, which allows a reworking of the gold mine with renewed methodologic capability. We may now anticipate considerable new inventiveness given this new capacity. A case in point is the Genta stain: as described herein it is a much-appreciated stain of high utility. However, one of the main constraints on widespread utility is that this excellent technique is very elaborate and difficult to perform, particularly in hospital practice. Nonetheless, with modern automation this technically trying method may become standard since automation should afford far better ease of use, as combinatorial chemistry would be a strength, not a liability. This promise is yet to be realized, but remains an excellent prospect in the ever-expanding special stain world.

Acknowledgments: The authors thank Marcus Goodwin for his technical assistance in manuscript preparation and Pru Mehta, Walter Sherman, Annie Pommerantz, and Chuck Lemme for their technical input.

REFERENCES

1. Grogan TM, Casey TT, Miller TP, et al. Automation of immunohistochemistry. In *Advances in pathology and laboratory medicine, Vol. 6*. St. Louis: Mosby. 1993:253–83.
2. Jackson TM, Ekins RP. Current practice and potential advantages of fluorescent Eu3+ chelates as non-radioisotopic tracers. *J Immunol Methods* 1986:87:13–20.
3. Verkman AS, Dix JA. Effect of unstirred layers on binding and reaction kinetics at a membrane surface. *Anal Biochem* 1984:142: 109–16.
4. Grogan TM, Rangel C, Rimsza L, et al. Kinetic-mode, automated double-labeled immunohistochemistry and in situ hybridization in diagnostic pathology. In: *Advances in pathology and laboratory medicine, Vol. 8*. St. Louis: Mosby. 1995:79–100.
5. Soans S, Galindo LM, Garcia FU. Mucin stain on frozen sections. *Arch Pathol Lab Med* 1999:123:378–80.
6. Egashira Y, Shimoda T, Ikegami M. Mucin histochemical analysis of minute gastric differentiated adenocarcinoma. *Pathol Inter* 1999;49:55–61.
7. Aslam A, Spicer RD, Corfield AP. Histochemical and genetic analysis of colonic mucin glycoproteins in Hirshsprung's Disease. *J Pediatr Surg* 1999:34:330–3.
8. Rothman C, Barshak I, Kopolovic J, Malik Z. Spectrally resolved morphometry of the nucleus of hepatocytes stained by four histological methods. *Histochem J* 1998:30:539–47.
9. Bozzo P, Vaught L, Alberts DS, Thompson D, Bartels PH. Nuclear morphometry in solar keratosis. *Analyt Quant Cytol Histol* 1998: 20:21–8.
10. Saiz E, Lubin J, Robinson MJ. The modified Steiner stain: a new use for an old stain? Staining cytomegalovirus-infected cells in gastrointestinal biopsies. *Histochem J* 1998:30:549–55.
11. McNally KJ, Peters A. A new method for intense staining of myelin. *J Histochem Cytochem* 1998:46(4):541–5.
12. Gores TJ, Leranth C, MacLusky NJ. The use of gold-substituted silver-intensified diaminobenzidine (DAB) and non-intensified DAB for simultaneous electron microscopic immunoperoxidase labeling o tyrosine hydroxylase and glutamine acid decarboxylase immunoreactivity in the rat medial preoptic area. *J Histochem Cytochem* 1992:34:1439–47.
13. Katoh R, Kakudo K, Kawaoi A. Accumulated basement membrane material in hyalinizing trabecular tumors of the thyroid. *Mod Pathol* 1999:12(11):1057–61.
14. Newman G, Jasani B, Silver development in microscopy and bioanalysis: a new versatile formulation for modern needs. *Histochem J* 1998:30:635–45.
15. Lamps LW, Bronner MP, Vnencak-Jones CL, et al. Optimal screening and diagnosis of microsporida in tissue sections: a comparison of polarization, special stains, and molecular techniques. *Am J Clin Pathol* 1998:109:404–10.
16. Franzon C, Muller A. Cryptosporidia and microsporidia - waterborne disease in the immunocompromised host. *Diagn Microbiol Infect Dis* 1999:34:245–62.
17. Didier E. Microsporidiosis. *Clin Infect Dis* 1998:27:1–8.
18. Dascomb K, Clark R, Aberg J, et al. Natural history of intestinal microsporidiosis among patients infected with human immunodeficiency virus. *J Clin Microbiol* 1999:37(10):3421–2.
19. Didier E, Orenstein JM, Aldras A. Comparison of three staining methods for detecting microsporidia in fluids. *J Clin Microbiol* 1995:33(12):3138–45.
20. Joste NE, Sax PE, Pieciak WS. Cytologic detection of microsporidia spores in bile: a comparison of stains. *Acta Cytol* 1999:43(2): 98–103.
21. Ryan NJ, Sutherland G, Coughlan K, et al. A new trichrome blue stain for detection of microsporidial species in urine, stool, and nasopharyngeal specimens. *J Clin Microbiol* 1993:31(12):3264–9.
22. Kokoskin E, Gyorkos TW, Camus A, et al. Modified technique for efficient detection of microsporidia. *J Clin Microbiol* 1994:32(4): 1074–5.

23. Bancroft JD, Stevens A, eds. *Theory and practice of histological techniques. 2nd ed.* New York: Churchill Livingstone, 1982.
24. Shoobridge MPK. A new principle in polychrome staining: a system of automated staining, complimentary to hematoxylin and eosin, and usable as a research tool. *Stain Technol* 1983;58(5):245–58.
25. Correa P. Helicobacter pylori and gastric carcinogenesis. *Am J Surg Pathol* 1995;19(suppl 1):S37–S43.
26. Isaacson PG. Recent developments in our understanding of gastric lymphomas. *Am J Surg Pathol* 1996;20(suppl 1):S1–S7.
27. Perez-Perez GI, Dworkin BM, Chodos JE, Blaser MJ. Campylobacter pylori antibodies in humans. *Annu Intern Med* 1988;109: 11–7.
28. Grahan DY. Benefits from elimination of H. pylori infection include major reduction in the incidence of peptic ulcer disease, gastric cancer, and primary gastric lymphoma. *Prev Med* 1994;23: 712–6.
29. Wotherspoon AC, Doglioni C, Diss TC, et al. Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue after eradication of H. pylori. *Lancet* 1993;342: 575–7.
30. Genta RM, Robason GO, Robinson DY. Simultaneous visualization of Helicobacter pylori and gastric morphology: a new stain. *Human Pathol* 1994;25:221–6.
31. El-Zimaity HM, Segura AM, Genta RM, Graham DY. Histologic assessment of Helicobacter pylori status after therapy: comparison of Giemsa, Diff-Quik, and Genta stains. *Mod Pathol* 1998;11(2): 288–91.
32. Laine L, Lewin DN, Naritoku W, Cohen H. Prospective comparison of H&E, Giemsa, and Genta stains for the diagnosis of Helicobacter pylori. *Gastrointest Endosc* 1997;45:463–7.
33. Cohen LF, Saveedudidib M, Phillips C, Shahab I. A new staining method for the identification of Helicobacter pylori and simultaneous visualization of gastric morphologic features. *Mod Pathol* 1997;10(11):1160–3.
34. Vartanian RK, Leung JK, Davis JE, Kim YB, Owen DA. A novel alcian yellow-toluidine blue (Leung) stain for Helicobacter species: comparison with standard stains, a cost-effectiveness analysis, and supplemental utilities. *Mod Pathol* 1998;11(1):72–8.
35. Doglioni C, Turrin M, Macri E, Chiarelli C, Bastianello G, Barbareschi M. HpSS: a new silver stain method for Helicobacter pylori. *J Clin Pathol* 1997;50:461–4.
36. Critchlow AJ. *Introduction to robotics*. Indianapolis, IN: Macmillan Publishing Co., 1985.

UNITED STATES DISTRICT COURT
DISTRICT OF MASSACHUSETTS

CIVIL ACTION NO. 04-11783-RWZ

CYTOLOGIX CORPORATION

v.

VENTANA MEDICAL SYSTEMS, INC.

MEMORANDUM OF DECISION

June 20, 2006

ZOBEL, D.J.

Plaintiff CytoLogix Corporation sued defendant Ventana Medical Systems, Inc. for infringement of U.S. Patent No. 6,541,261 B1 (the "'261 patent") and U.S. Patent No. 6,783,733 B2 (the "'733 patent"). These patents concern "technology for staining tissue samples in connection with the diagnosis of diseases such as cancer." (Pl.'s Mem. in Support of Summ. J. and Claim Construction 1). Plaintiff accuses defendant's Benchmark instrument product line of infringing these patents and now moves for claim construction and summary judgment on claims 1 and 2 of the '261 patent. Defendant opposes.

The first claim of the '261 patent describes a method for processing samples mounted on microscope slides that are placed on a platform. See '261 Patent 12:14–25. In addition to other characteristics, the method provides for "moving the platform and a liquid dispenser relative to each other." Id. at 21-22. The second claim of the '261 patent depends from claim 1 and describes the same method with the

distinction that "each heating element heats only one slide." Id. at 26-28. The parties dispute the meaning of the language, "moving the platform and a liquid dispenser relative to each other," and each party bases its summary judgment argument on its proposed claim construction. According to plaintiff, the disputed language means that "[t]here is relative movement between the platform and the liquid dispenser. Relative movement may be accomplished by moving the platform, or the liquid dispenser, or both." (Pl.'s Mem. in Support of its Combined Mot. 7). Defendant, on the other hand, urges that "[t]his claim language requires moving both 'the platform' and 'a liquid dispenser.' It does not permit moving only one of them." (Def.'s Mem. in Opp. to Pl.'s Combined Mot. 6). Giving rise to the dispute are certain of defendant's slide processing products that – solely for purposes of arguing the instant motion, the parties agree – involve a mobile liquid dispenser but a stationary platform.

"[P]atent infringement analysis involves two steps: claim construction, and application of the construed claim to the accused product or process." Wilson Sporting Goods Co. v. Hillerich & Bradsby Co., 442 F.3d 1322, 1326 (Fed. Cir. 2006). The first step, claim construction, requires that the Court construe "only those terms . . . that are in controversy, and only to the extent necessary to resolve the controversy." Vivid Technologies, Inc. v. American Science & Engineering, Inc., 200 F.3d 795, 803 (Fed. Cir. 1999). "[T]he words of a claim 'are generally given their ordinary and customary meaning,'" in other words, "the meaning that the term would have to a person of ordinary skill in the art in question at the time of the invention, i.e., as of the effective filing date of the patent application." Phillips v. AWH Corp., 415 F.3d 1303, 1312-13

(Fed. Cir. 2005). A disputed claim term may be interpreted according to "'the words of the claims themselves, the remainder of the specification, the prosecution history, and extrinsic evidence concerning relevant scientific principles, the meaning of technical terms, and the state of the art." Id. at 1314.

Plaintiff relies, first, on the plain language of the disputed claims and argues that the phrase, "relative to each other," requires only that either the platform or the dispenser move, since either event would create relative movement. As defendant argues, however, this interpretation negates the conjunctive term "and," as used in the phrase, "moving the platform and a liquid dispenser relative to each other." Plaintiff's expert, Professor Alexander H. Slocum, explains the term "and" from the perspective of an individual with ordinary skill in the art – for example, an engineer with basic training in physics – as creating reciprocal frames of reference. The platform is the frame of reference for whether the dispenser moves, and the dispenser is the frame of reference for whether the platform moves. (See Slocum Aff. ¶ 9). Even if, in fact, the platform is static and only the dispenser moves, a tiny observer standing on the platform who sees the moving dispenser would not know whether to attribute the motion she observes to the platform or the dispenser. Thus, at least in theory, both the dispenser and the platform are moving relative to each other. The perspective is similar to that of a passenger who sits on a stationary northbound train next to a stationary southbound train on adjacent tracks. When one or both of the trains begin moving, the passenger will comprehend motion but will not know whether it is his train alone, the southbound train alone or both trains together that are moving.

The difficulty with this position is that it does not account for a third frame of reference created by the requirement that something or someone be "moving" the platform or dispenser. In describing a method of moving the platform or dispenser, the claim language adopts the perspective of the person or thing responsible for causing this movement, not the theoretical perspective of an observer sitting on either the platform or dispenser. While such a theoretical observer may be unable to discern whether movement comes from the dispenser or the platform, the person or thing responsible for moving the platform and dispenser will have this knowledge.

Defendant's expert, Professor Geoffrey Nunberg underscores this understanding from the view of a linguist interpreting the disputed claim language according to rules of grammar. However, "one of ordinary skill in the art" generally refers to an individual with expertise in the field of the patented invention, not someone skilled in the field of language and drafting. See Phillips, 415 F.3d at 1333 (explaining that "[f]or each patent, for example, who qualifies as one of ordinary skill in the art will differ, just as the state of the art at the time of the invention."). Thus, while interesting, Professor Nunberg's testimony is not representative of a person of ordinary skill in the art, and I do not rely on his opinion in resolving the instant dispute.

Plaintiff next argues that its interpretation of the language in claim 1 is more consistent with the articulation of dependent claims in the '261 patent. Plaintiff focuses on claim 3 that describes a "method of processing samples . . . wherein the platform is a moving platform capable of indexing slides adjacent to a stationary liquid dispensing location." '261 Patent 12:29-32. According to plaintiff, depiction of the platform as

"moving" necessarily implies that the platform may otherwise be immobile. Defendant counters that the purpose of claim 3 is not to identify the platform as moving, but to provide that it be "capable of indexing slides adjacent to a stationary liquid dispensing location." Defendant also relies upon claim 7 that references "said moving platform" and, thereby, implies that the "said" platform portrayed in claim 1 is mobile. Plaintiff asserts that this language resulted from a clerical error that should have been, and will eventually be, amended. "An error in the prosecution record must be viewed as are errors in documents in general; that is, would it have been apparent to the interested reader that an error was made, such that it would be unfair to enforce the error." Biotec Biologische Naturverpackungen GmbH & Co. KG v. Biocorp, Inc., 249 F.3d 1341, 1348 (Fed. Cir. 2001). Because nothing suggests that an interested reader would have understood the inclusion of the term "said" to be in error, claim 7 should be read as originally drafted.

The patent specification further supports defendant's position, as the parties agree that none of the preferred embodiments describe a stationary platform. Plaintiff correctly notes that the embodiments do not foreclose the possibility of a stationary platform, but they certainly do not support an interpretation of the claim language as describing a stationary platform. While the Federal Circuit "ha[s] repeatedly warned against confining the claims to [very specific] embodiments" and "strictly limiting the scope of the claims to the embodiments disclosed in the specification," it has also discouraged "divorcing the claim language from the specification." Phillips, 415 F.3d at 1323-24. Appropriate interpretation will consider the full context of the patent. See id.

Turning from the platform, plaintiff focuses on the first preferred embodiment's description of the liquid dispenser. The embodiment describes a stationary hammer that helps push liquid from the dispenser onto a slide. See '261 Patent 5:25-51. In order to define the dispenser as including a stationary element, plaintiff characterizes the hammer as part of the liquid dispenser. However, the patent specification defines the hammer as part of the dispensing station, not the dispenser, and plaintiff offers no rationale for a different characterization. See id. 5:25-26 (providing that "the dispensing station comprises a soft hammer . . .").

The file history of the '261 patent also favors defendant. It reveals amendments to the language in claim 1 that include replacing the term "moving platform" with "moving the platform and a liquid dispenser relative to each other." (Pl.'s Mem. in Support of Summ. J. and Claim Construction 10-11). Plaintiff believes that this change underscores the importance of relative motion and the ability to accomplish such motion between the platform and the liquid dispenser without having a moving platform. (Id.). This understanding is inconsistent, however, with the repeated description of "moveable slides" and the use of a carousel in describing the platform, as cited by defendant. (See Def.'s Mem. in Opp. to Pl.'s Combined Mot. 19-24). Plaintiff argues that independent slide heating, and not movability, constituted the Examiner's focus on the patent, so that use of these terms occurred only in passing, not deliberately. Even assuming arguendo that plaintiff is correct, "a patentee's statements during prosecution, whether relied on by the examiner or not, are relevant to claim interpretation." Microsoft Corp. v. Multi-Tech Systems, Inc., 357 F.3d 1340, 1350 (Fed.

Cir. 2004). Plaintiff also posits that the term moveable "was appropriate, given that the movement had already [sic] been defined as relative to the liquid dispenser." (Pl.'s Mem. in Support of Summ. J. and Claim Construction Footnote 4). This reasoning cannot provide additional support for plaintiff's position, because it necessarily presumes that plaintiff's argument for relative motion would prevail and thus is circular.

In light of the applicable legal standard, the parties' written submissions, and the argument of counsel, I construe the disputed claim language as follows:

Term	**Court's construction**
Moving the platform and a liquid dispenser relative to each other	Moving both the moveable platform and a moveable liquid dispenser relative to each other

Plaintiff's Motion for Summary Judgment (#42 on the docket) is denied.

06/20/06
DATE

/s/ Rya W. Zobel
RYA W. ZOBEL
UNITED STATES DISTRICT JUDGE